

# High Prevalence of *Mycoplasma faucium* DNA in the Human Oropharynx

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***Mycoplasma faucium* has recently been associated with brain abscesses and seems to originate from the mouth. We evaluated its prevalence by quantitative real-time PCR (qPCR) in the oropharynxes of 644 subjects and found that 25% harbored *M. faucium*, probably constituting the gateway for entrance of the bacteria into cerebral abscesses.**

*Mycoplasma* spp. are ubiquitous bacteria characterized by their lack of cell wall. To date, 124 validated species have been described (List of Prokaryotic names with Standing in Nomenclature [<http://www.bacterio.net/>]), which colonize humans and/or animals. Among these fastidious bacteria, some species are known to be pathogenic for humans, such as *Mycoplasma pneumoniae*, which is responsible for respiratory diseases, along with *Mycoplasma genitalium* and *Mycoplasma hominis*, which are responsible for genital infections. Other species are considered to be commensals of the respiratory or genital tract (1).

*Mycoplasma faucium* was described in 1969 by Fox et al. as a rare inhabitant of the human oropharynx under the name *Mycoplasma orale* type 3 (2). In 1974, Freundt et al. proposed the name *M. faucium* based on the Latin word fauces meaning the throat (3). This bacterium was regarded as a commensal of the human oral flora and as nonpathogenic until 2009 when it was identified for the first time by 16S rRNA gene metagenomics in the brain abscesses of three patients (4). One additional case was found in a further study in 2012 (5). In all, *M. faucium* DNA has been detected in the cerebral abscesses of 4/51 (8%) patients (4, 5). The bacterium was significantly associated with polymicrobial abscesses and with patients presenting with sinusitis or a dental defect (5).

Brain abscesses are a focal infection and in 25% to 50% of cases are the result of direct extension from a contiguous suppurative focus such as mastoiditis, sinusitis, otitis, or from bacteria present in the mouth (6). Although *M. faucium* has recently been associated with brain abscesses, there are currently no accurate data on its prevalence in the human oral cavity. Our objective was to evaluate the prevalence of *M. faucium* carriage in the human oropharynx.

Between January 2013 and December 2014, 644 people were enrolled on a voluntary basis into four different cohorts of patients. Of these patients, 293 presented with tonsillitis, 23 presented with meningitis, 110 presented with acute diarrhea, and 218 were pilgrims sampled before departing from Marseille, France to Mecca (7). All of the cohorts were approved by our local ethics committee under numbers 1301 (tonsillitis cohort), 1303

(meningitis cohort), 1305 (diarrhea cohort), and 1356 (pilgrims cohort) and by the French National Drugs and Health Products Agency under numbers 2012-A01593-40, 2012-A01591-42, 2012-A01590-43, and 2013-A00961-44, respectively. Written informed consent was obtained from all participants. One pharyngeal swab ( $\Sigma$ -Transwab, Sigma) was collected from each patient. DNA was extracted using the QIAamp tissue kit according to the manufacturer's recommendations (Qiagen, Courtaboeuf, France). Specific quantitative real-time PCR (qPCR) targeting the internal transcribed spacer (ITS) region for the detection of *Mycoplasma* spp. and the *rpoB* gene for the detection of *M. faucium* was used with primers and TaqMan probes described previously (8). qPCR was performed with the QuantiTect Probe PCR kit (Qiagen) and by using a CFX96 thermocycler (Bio-Rad, Marne-la-Coquette, France). Synthetic positive controls (108 bp from the *rpoB* gene and 114 bp from the ITS region) produced with a pUC57 plasmid were used, and sterile water was used as a negative control. The specificities of the primers and probes were verified *in silico* by conducting a BLAST search in GenBank and by performing qPCR on purified genomic DNA from a panel of 77 bacterial strains (including closely related bacterial strains and bacteria commonly present in the respiratory tract) (see Table S1 in the supplemental material). To test the sensitivity of the method, 10-fold dilutions of plasmid DNA standards were performed to obtain final concentrations

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ranging from 1 to  $10^9$  DNA copies/ $\mu$ l. Each dilution was tested by qPCR in duplicate, and a standard curve was generated (see Fig. S1 in the supplemental material). The limit detection was 1 DNA copy/ $\mu$ l for qPCR targeting *Mycoplasma* spp. and *M. faucium* (see Table S2 in the supplemental material). All samples with threshold cycle ( $C_T$ ) values of  $\leq 38$  were considered positive. The DNA extraction quality was verified by qPCR targeting of the human beta-actin gene (8). A fragment of the *rpoB* gene of the *Mycoplasma* spp. was amplified by PCR and was sequenced with two primer sets targeting the *M. pneumoniae* and *M. hominis* groups as previously described (9).

The participants included 342 women (53%) and 302 men (47%) with a median age of 28 years (range, 0 to 92 years). Among the 644 swabs tested, 283 (44%) were positive for *Mycoplasma* spp. and 163 (25%) were positive for *M. faucium*. All of the samples that were positive for *M. faucium* were also positive for *Mycoplasma* spp. For the cohort of patients presenting with tonsillitis (median age, 5), 12/293 (4%) were positive for *M. faucium*, 2/23 (9%) were positive from the cohort of patients presenting with meningitis (median age, 17), and 24/110 (22%) were positive from the cohort of patients with diarrhea (median age, 41). The prevalence (57%, 125/218) was significantly higher among pilgrims (median age, 62) than it was in the other cohorts ( $P < 0.0001$  using the  $\chi^2$  test). The presence of *M. faucium* in the pharynx was influenced by age and sex. The prevalence of *M. faucium* carriage was significantly lower in children and adults under the age of 25 years (3%, 8/307) compared with that in adults over the age of 25 years ( $P < 0.0001$  using the  $\chi^2$  test). The prevalence of *M. faucium* was 35% (23/65) in adults between 25 and 45 years old, and it reached 49% (132/272) in adults over the age of 45 years. *M. faucium* carriage was higher in women (28%, 98/342) than it was in men (21%, 65/302), ( $P = 0.037$  using the  $\chi^2$  test). Nineteen percent of the patients demonstrated carriage of a *Mycoplasma* spp. other than *M. faucium*. We sequenced 10 of these samples and showed the presence of *Mycoplasma* species commonly found in the oral cavity (1). *Mycoplasma salivarium* was identified with 99% similarity with the GenBank sequence in eight samples, and *Mycoplasma orale* was identified with 99% similarity in two samples. Twenty samples harbored a higher number of DNA copies of *Mycoplasma* spp. compared to that of *M. faucium*, suggesting that several *Mycoplasma* species could be present simultaneously in the oropharynx.

Our study highlights *M. faucium* as a common bacterium in the oral cavities of adults. Until now, its prevalence in the pharynx has never been accurately assessed. *Mycoplasma* is difficult to culture and its detection requires the use of molecular tools. qPCR is routinely performed in our laboratory, and we employed strict and validated protocols, including positive and negative controls, to validate the qPCR assay (8). Our in-house qPCR test for detecting *M. faucium* is used routinely on brain abscesses, with positive results in 6/210 specimens since 2011 (8). In five cases, *M. faucium* was associated with a polymicrobial infection (two with *Staphylococcus aureus*, two with *Streptococcus intermedius*, and one case with *Pseudomonas aeruginosa*). Including previously reported cases (4, 5), a total of 10 patients harbored *M. faucium* in brain abscesses. The median age was 50 years (range, 11 to 76 years), and 60% of the patients were male, which is consistent with the results reported herein; more frequent carriage of *M. faucium* was found in elderly adults. In each of the different cohorts, age seems to be a risk factor for *M. faucium* carriage. In fact, the median age of

patients positive for *M. faucium* was 29 for the tonsillitis cohort, 38 for the meningitis and diarrhea cohorts, and 60 for the pilgrims cohort. However, a bias exists between the different cohorts concerning the geographical origin of the patients, particularly for the cohort of pilgrims that include a majority of Muslims. But a higher prevalence of *M. faucium* in adults over the age of 25 years was found independently for the four different cohorts. The geographical origin and specific habits of people may also influence *M. faucium* carriage; larger studies are needed to confirm this hypothesis.

The oropharynx probably constitutes the entry site of bacteria into a cerebral abscess. The meaning of the presence of this bacteria in brain abscesses is unclear. In fact, the pathogenicity of *M. faucium* in brain abscesses is questionable because it has been found only in polymicrobial infections, and patients recovered without specific treatment (5) as the bacterium is resistant to the empirical antibiotherapy commonly used to treat these infections (10). However, despite the diversity of *Mycoplasma* species commonly present in the oral cavity (1), only *Mycoplasma salivarium* (11) and *M. faucium* have been found in brain abscesses, which suggests a specific pathogenic role of these bacteria. However, there were several limitations in our study: we included only one city (Marseille) and employed a limited sample size. Further studies are needed to better understand the role of *M. faucium* in cerebral abscess and to more accurately characterize pharyngeal carriage in larger populations, along with seasonal variations.

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